2500 Old House Point Road• Fishing Creek, MD 21634• Phone: 410-397-3664
E-Mail: ricky@cgoysters.com Web: www.hioac.com

Date: July 29, 2014

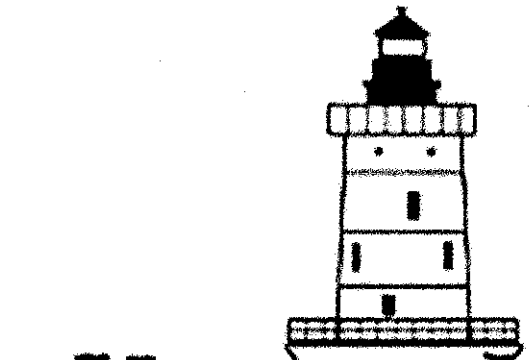
Ken B. Moore
Executive Director
Interstate Shellfish Sanitation Conference
209-2 Dawson Road
Columbia, South Carolina 29223-1740

To Whom It May Concern:

Enclosed is Hooper's Island Oyster Aquaculture Comapany's response to the Interstate Shellfish Sanitation Conference (ISSC) Request for Proposal regarding techniques and practices for Vibrio Reduction on July 21,2014. We would like to thank you for the opportunity to submit a proposal and hope to have your consideration for the project proposed.

Sincerily,

Ricky Fitzhugh
President
Hoopers Island Oyster Aquaculture Company
2500 Old House Point Road
Fishing Creek, MD 21634



Hoopers Island

Oyster Aquaculture Co.

The effect of temperature and salinity on *Vibrio parahaemolyticus* depuration in the Eastern oyster *(Crassostrea virginica)*

Interstate Shellfish Sanitation Conference (ISSC) Request for Proposal Response

July 30, 2014

Executive Summary

Hooper's Island Oyster Aquaculture Company (HIOAC) is designed to provide the infrastructure around oyster aquaculture to support a new approach around traditional methods used by watermen. HIOAC is the leader in the state of Maryland around new aquaculture technology while manufacturing a line of equipment specialized to advance a growing industry. We believe that with our knowledge of the oyster industry coupled with established technology already being used at HIOAC, we can produce a method that is effective and can be applied across an entire industry to reduce *Vibrio* in live oysters.

With the consumption of raw oysters steadily becoming more popular around the country, the number of shellfish related illnesses is increasing as well. The need for an effective post-harvest processing system that can be applied across a growing industry is becoming more evident. While there are effective post-harvest processing techniques accepted by the FDA and other agencies, they all result in killing the product. While these systems are useful in treating oysters that will be processed, live oysters are more desirable to the half-shell market.

In this study, HIOAC proposes that controlling water temperature and salinity, post-harvest, will reduce the *Vibrio* load in live oysters. While there has been multiple studies done to show the effect of water temperature on *Vibrio*, much less is known about the effect that salinity change has on *Vibrio* survival. Introducing infected oysters to temperatures that inhibit *Vibrio* production while not causing inactivity in the oyster, will allow the oyster to purge bacteria found in the shell and within their meat. Any purged bacteria found in the water column will be killed by UV-C light and removed by fractionation.

The same method will be used to test the effectiveness of salinity on *Vibrio* load in live oysters. Although *Vibrio* is found across a wide range of salinities, a drastic change in the ambient salinity they are found in may have a direct impact on the reduction of *Vibrio* in live oysters. Environmental conditions are an important factor in any healthy population of terrestrial and marine organisms; thus, altering

environmental conditions could have a drastic impact on the health of *Vibrio* populations.

Scope, Approach, and Methodology

Preparation of Vibrio parahaemolyticus cultures

V. parahaemolyticus isolated from infected oysters and stored in the UMES Food Safety Lab in Princess Anne, Maryland will be used in this study.

Oyster Preparation

Cage cultured triploid oysters (*Crassostrea virginica*) grown in the Chesapeake Bay, Maryland will be used for this assessment. Prior to the introduction of *V. parahaemolyticus*, oysters will be washed to remove sediment and stored for 12 hours at 5° C. Oysters will be placed in artificial seawater (ASW) contaminated with *V. parahaemolyticus* at ambient conditions overnight to allow for accumulation of *Vibrio*. Oysters will be tested prior to treatment to determine initial *Vibrio* levels.

Depuration of Oysters

Infected oysters will be treated in a commercial recirculating tank equipped with UV-C light, a protein skimmer, water chiller and biological filter. Oysters will be tested for *Vibrio* at 0, 6, 12, 24, and 48 hours over the course of the study. Temperature and salinity will be tested independently as well as collectively. Effectiveness of temperature on *Vibrio* will be tested at ambient salinity and 15° C initially. Oysters will be held at 15° C for half of the indicated treatment time before water temperatures are reduced. For example, oysters exposed for 12 hours will be held at 15° C for 6 hours before water temperatures are reduced over the next 6 hours (Table 1). Salinity effectiveness will be tested at ambient water temperatures with an increased salinity of 26 ppt. Temperature and salinity will be tested collectively at 15° C with a reduction to 10° C as stated in the independent temperature assessment with a salinity of 26 ppt.

Exposure Time (Hours)		Temperature (15° C ->10° C)	Salinity (26 ppt)	Temperature + Salinity (15° C -> 10° C @ 26 ppt)
0				
6				
12				
24				
48				

Table 1.

Bacterial Analysis

Initial and final microbiological testing will be done using MPN-BAX method outlined in FDA BAM (2004) at UMES Center for Food Science and Technology Lab in Princess Anne, MD.

Project Deliverables

We expect to deliver a written report with a statistical analysis showing the reduction of *Vibrio parahaemolyticus* in live oysters on a commercial scale to meet standards set forth by the USFDA and the ISSC.

Project Management Approach

Each phase of this project will be handled using appropriate laboratory and research techniques by experienced individuals. Oysters will be harvested by Hooper's Island Oyster Aquaculture Company in Fishing Creek, MD. Prior to

exposing oysters to *V. parahaemolyticus*, oysters will be held at temperatures less than 7° C (45° F). Any oysters used for testing will be thoroughly cleaned to remove sediment and other possible contaminants before processing begins. After accumulation of *V. parahaemolyticus* is complete, testing will be done in wet storage system designed to regulate and maintain temperature and salinity to desired levels at the Hooper's Island Oyster Aquaculture Company Facility located in Fishing Creek, MD (see appendix III). Following treatment, oysters will be transported to the University of Maryland – Eastern Shore in Princess Anne, MD via refrigerated van at temperatures < 7° C (45° F) for testing. All oysters will be chilled to < 10°C (<50° F) prior to transport to reduce propagation of *V. parahaemolyticus.*

Detailed and Itemized Pricing

ISSC Contribution

	Product	Cost
Water Quality Monitoring Equipment	YSI 5200A Water Quality Monitoring System	$1,925.00
	4-M Cable w/ D.O/Temp/Conductivity	$1,015.00
	pH/ORP Kit	$360.00
	Conductivity / Temperature Probe	$430.00
	Rail Mount Kit	$42.00
	Aqua manager Software	$410.00
Water Chiller/Heater	HP53 Heat Pump	$8,735.00
	Water Pump	$750.00
	Installation/Temperature Controls	$5,000.00
V.p Testing / Lab Fees	Testing	$9,000.00
	Total Budget	**$27,667.00**

Company Match

	Labor	$12,000.00
	Equipment and Supplies	$11,000.00
	Travel (Delivery of samples to lab)	$4,500.00
	Total Budget	**$27,500.00**

Appendix I. References

Chae, M. J., Cheney, D., & Su, Y. C. (2009). Temperature effects on the depuration of Vibrio parahaemolyticus and Vibrio vulnificus from the American oyster (Crassostrea virginica). *Journal of food science, 74*(2), M62-M66.

Phuvasate, Sureerat, Ming-Hui Chen, and Yi-Cheng Su. "Reductions of Vibrio parahaemolyticus in Pacific oysters Crassostrea gigas by depuration at various temperatures." *Food microbiology* 31.1 (2012): 51-56.

Shen, X., Cai, Y., Liu, C., Liu, W., Hui, Y., & Su, Y. C. (2009). Effect of temperature on uptake and survival of Vibrio parahaemolyticus in oysters Crassostrea plicatula. *International journal of food microbiology, 136*(1), 129-132.

Appendix II. Project Team Staffing

Dr. Chengchu (Cathy) Liu, Ph.D. –Seafood Technology Specialist, UMES Center for Food Science and Technology

- Dr. Liu has extensive experience working with *Vibrio spp.* as well as various species of oysters. Dr. Liu's focus in research is various depuration techniques of *Vibrio parahaemolyticus* in live oysters. Dr. Liu has been recognized for her research, being honored by Phi Tau Sigma as a lifetime member as well being IFT Professional Member of Institute of Food Technologist.

Chanelle White, M.S – Laboratory Technician, University of Maryland – Eastern Shore

- Chanelle has worked in many fields including agriculture and food microbiology. During her time as a research specialist at UMES, she worked on projects such as isolating *V. vulnificus* and *V. parahaemolyticus* from oysters and isolating *L. monocytogenes* from crabmeat. She has a strong professional background in food science, microbiology and molecular biology.

Jordan Shockley, B.S – Nursery / Farm Manager, Hooper's Island Oyster Aquaculture Company

- Jordan gained experience working in a hatchery while in college; working at University of Maryland Center for Environmental Science - Horn Point Oyster Hatchery. His interests include ways to increase setting efficiency of oyster larvae and depuration techniques of *V. parahaemolyticus* including temperature and salinity effects and treatments with bacteriophage.

Ricky Fitzhugh, B.S – President, Hooper's Island Oyster Aquaculture Company

- Ricky began his career in seafood after his time at the University of Maryland. He started Terrapin Fish Company, a wholesale seafood distribution company. He then founded and developed Rosedale Ice Company, with multiple locations and USAICE, an ice sculpting company catering to large corporate events. After co-founding HIOAC, Ricky turned his focus to establishing the safest raw oyster on the market.

Johnny Shockley – Vice President, Hooper's Island Oyster Aquaculture Company

- Johnny is a 3rd generation, lifetime waterman and business man; harvesting crabs, fish and oysters commercially while owning and operating Chesapeake Treasures, a seafood Carry-out for 22 years, before co-founding HIOAC. Johnny has extensive experience in wood and fiberglass fabrication, building his first boat at the age of 18 and designing a line of equipment currently manufactured by Hooper's Island Oyster Aquaculture Company.

Appendix III. Company Overview

Hooper's Island Oyster Aquaculture Company
DUNS # 07-879-0830
2500 Old House Point Road
Fishing Creek, MD 21634
Tel. 410-397-3664
Fax. 410-397-3634

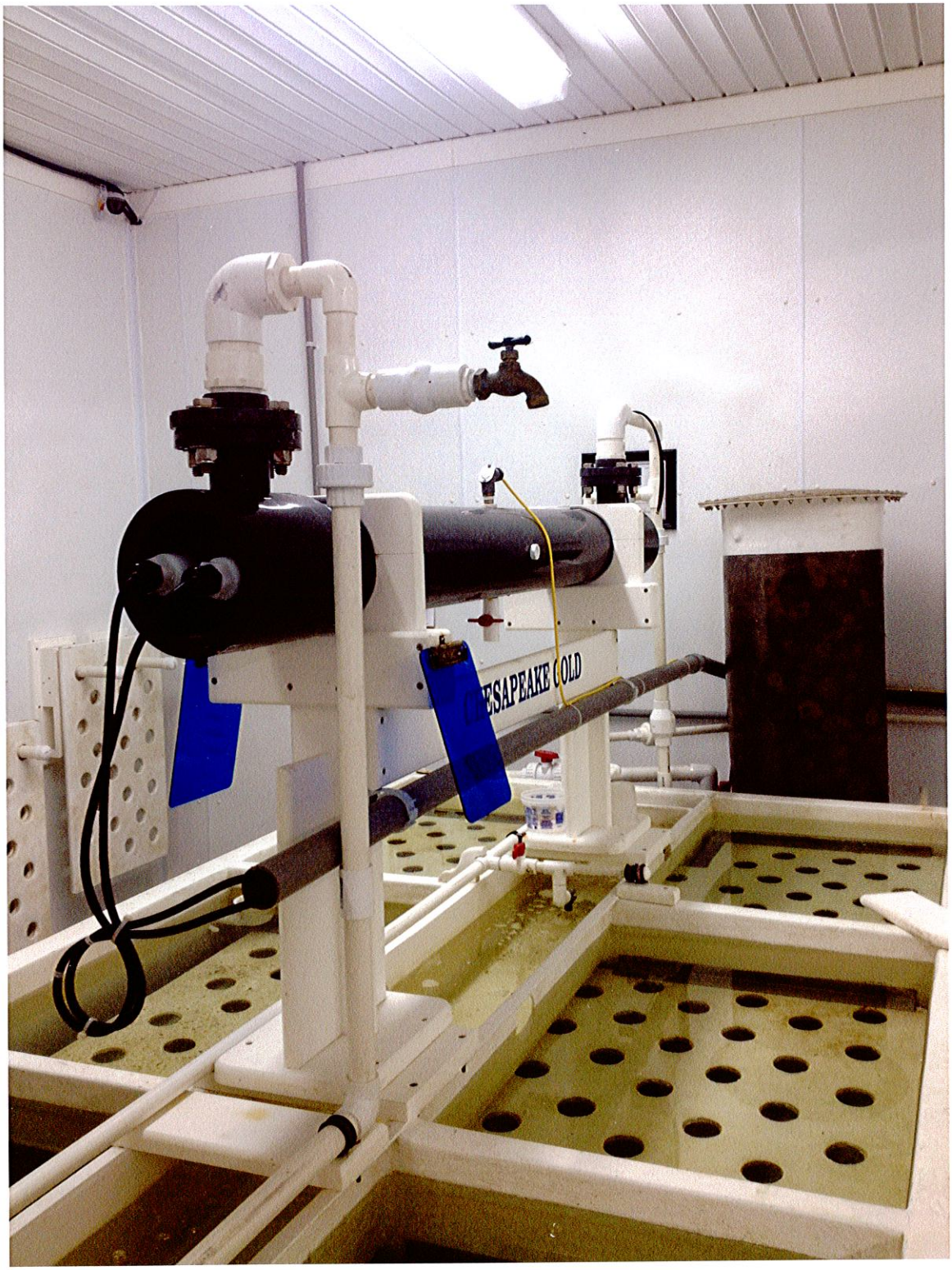
Hooper's Island Oyster Aquaculture Company (HIOAC) was established in 2009 as one of the first oyster aquaculture companies in the State of Maryland under the new laws passed by Governor Martin O'Malley. HIOAC currently manufactures oyster aquaculture equipment while growing 2 brands of oysters, *Chesapeake Gold Oysters* and *Holy Grail Oysters.* HIOAC is interested in adding value to their products through aquaculture technology through partnerships with Maryland Industrial Partnership and the University of Maryland. Currently, we have completed one project, with another in the development stages. In partnership with the University of Maryland and Maryland Industrial Partnership, HIOAC developed a state-of-the-art wet storage system capable of regulating salinity while maintaining a sterile environment. We are interested in testing the capability of depurating oysters of *Vibrio spp.* as well as other bacteria in this system to increase the safeness of consuming raw oysters. HIOAC in partnership with University of Maryland is currently developing a grading system capable of grading oysters based on length, width, depth and meat quality. We are interested in testing the capability of depurating oysters of *Vibrio spp.* as well as other bacteria in this system to assure the invulnerability of consuming raw oysters.

Conflicts of Interest

We have extensive experience working with other parties. We do not anticipate any conflicts of interest, however, in the case conflicts do arise, we will use previous problem solving techniques utilized in previous scenarios throughout our 16 years experience in other business ventures. We are willing to work all parties to resolve any issues that may arise over the span of this project. We are willing to work with all parties for this project.

Contact/person to contractually bind the organization against proposal:
Ricky Fitzhugh
President
Hooper's Island Oyster Aquaculture Company
2500 Old House Point Road
Fishing Creek, MD 21634
Tel. 410-397-3664
Direct. 410-463-0848

Equipment / Facility


ESAPEAKE COLD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